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Long distance effect on ligand-gated ion channels extracellular domain may affect interactions with the intracellular machinery

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Modulation of receptor trafficking is critical for controlling neurotransmission. A $\gamma 2(R43Q)$ point mutation on GABA_A receptor subunit is linked to epilepsy in human. We recently analyzed the effect of this amino-acid substitution on GABA_A receptor trafficking and showed that this mutation as well as agonist application, both affecting GABA_A receptor extracellular domain, have an effect on receptor endocytosis. By comparing homology models based on ligand gated ion channels in their active and resting states, we reveal that the $\gamma 2R43$ domain is located in a loop that is affected by motion resulting from receptor activation. Taken together, these results suggest that endocytosis of GABA_A receptors is linked to agonist induced conformational changes. We propose that ligand or modulator binding is followed by a whole chain of interconnections, including the intracellular domain, that may influence ligand-gated channel trafficking.

Fast neurotransmission relies greatly on ligand-gated ion channels (LGICs) that are involved in many physiological functions. Plasticity of neurotransmission in physiological and pathophysiological states depend on LGIC trafficking that is modulated by a number of different mechanisms including surface targeting, mobility, and endocytosis.¹⁻³ GABA_ARs involved in fast inhibitory neurotransmission are also regulated by the exchange between surface and intracellular compartments via a constitutive clathrin-mediated dynamin-dependent endocytosis

pathway.⁴ This constitutive internalization is modulated by intracellular mechanisms and is altered in pathological conditions.⁵⁻⁹ Other LGIC such as AMPA or P2X receptors are also constitutively internalized by a clathrin-dependent endocytosis. In contrast, NMDA receptors are stabilized via an interaction with PDZ domain proteins and are more stable at the postsynaptic membrane.¹⁰ Among P2X subtypes, P2X4-containing receptors are specifically and constitutively internalized.¹¹

Genetic evidence has revealed a direct link between epilepsy and GABA_ARs dysfunction, including trafficking alteration.¹²⁻¹⁴ These mutations also offer an opportunity to obtain new insights into GABA_AR structure and function.¹⁵ For example, an R43Q mutation located in the $\gamma 2$ -subunit N-terminal extracellular domain (Fig. 1A) is linked to childhood absence epilepsy and febrile seizure.^{13,14} We recently analyzed $\gamma 2(R43Q)$ trafficking and showed that the $\gamma 2$ subunit containing the R43Q mutation increased a clathrin- and dynamin-dependent endocytosis of GABA_ARs, hindering their detection on the cell surface.¹⁶ The $\gamma 2(R43Q)$ -dependent endocytosis was reduced by GABA_ARs antagonists gabazine and picrotoxin, acting at a different site suggesting an allosteric effect i.e., that $\gamma 2(R43Q)$ -containing GABA_ARs are in a conformational state that promotes internalization. Further experiments revealed that agonist exposure triggered an increase of wild-type GABA_AR endocytosis, both on native- and recombinant-GABA_ARs.¹⁶ Application of their respective agonists enhances also

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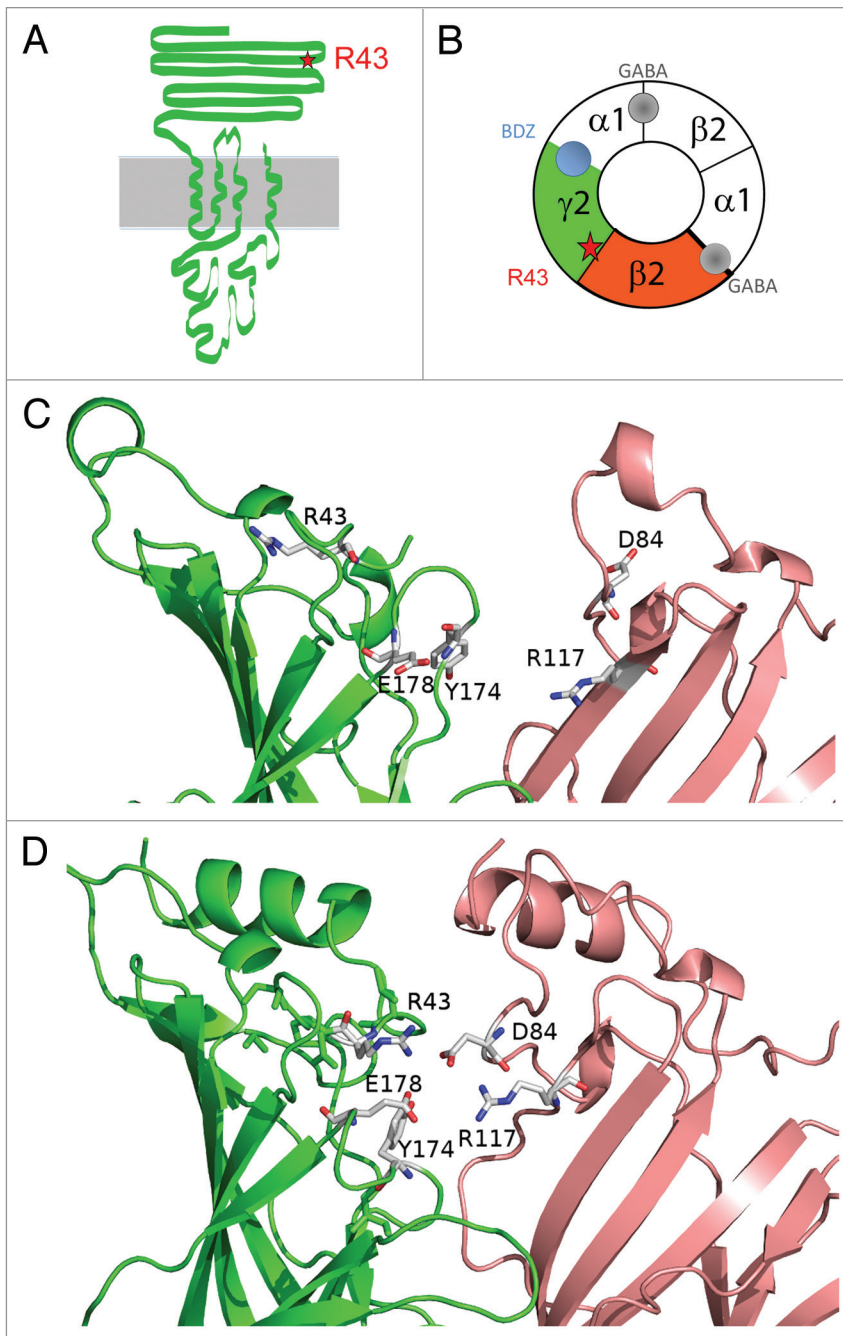


Figure 1. The $\gamma 2(R43Q)$ mutation destabilize the open-channel conformation. **(A)** Schematic representation of $GABA_A$ subunits showing a large N-terminal extracellular domain, 4 transmembrane domains and a large cytoplasmic loop. The location of the $\gamma 2(R43Q)$ point mutation associated with childhood absence epilepsy and febrile seizure is indicated. **(B)** Schematic diagram of a $\alpha 1\beta 2\gamma 2$ $GABA_A$ which illustrates the 5 combined subunits that form the complex, the 2 GABA active binding sites at the $\beta 2$ and $\alpha 1$ interfaces (gray circles) and the benzodiazepine (BDZ, blue circle) allosteric binding site at the $\alpha 1$ and $\gamma 2$ interface. In current models, $\gamma 2R43$ (red star) is at the interface with $\beta 2$. **(C,D)** Model of a $\alpha 1\beta 2\gamma 2$ $GABA_A$ viewed from the outside. Here only the $\gamma 2$ and $\beta 2$ subunits are shown for clarity ($\gamma 2$ in green and $\beta 2$ in pink, see **(B)**). $\gamma 2$ (R43, Y174, E178) and $\beta 2$ (D84, R117) residues are represented by sticks. These residues are within loops identified as being involved in the channel-opening motion: **(C)** resting state model, **(D)** active state model.

the rate of AMPA- or P2X4-receptor internalization suggesting that the increase might be caused by an agonist-induced

conformational change in the receptor promoting an interaction with components of the endocytotic machinery.¹¹

The $GABA_A$ receptor extracellular domain contains binding sites for agonists and allosteric modulators (Fig. 1B), while the intracellular domain mediates interactions with trafficking factors.^{2,6,8,17} Consequently, our findings¹⁶ suggest that $GABA_A$ receptor internalization is driven by a global conformational change. Molecular models show that the $\gamma 2R43$ residue is at the $\gamma 2/\beta 2$ interface in the extracellular domain, on a loop positioned above the pocket, which is homologous to the GABA binding sites. Interestingly, many mutations in nicotinic receptors linked to diseases are at the interface between receptor subunits;¹⁸ they alter the gating allosterically, i.e., from a distance.^{18,19} A model indicates that $\gamma 2R43$ and $\gamma 2E178$ are connected through a bifurcated salt bridge: this model has been used to study the $\gamma 2(R43Q)$ mutation.^{12,20,21} One of these studies has suggested that these positions have a long-range allosteric effect.²⁰ In our new $GABA_A$ receptor model derived from the Glutamate-gated chloride channel in the active state recently published,²² the R43 residue of the $\gamma 2$ -subunit is connected to Y174 and E178 from the loop B and to $\beta 2$ subunit via polar interactions that should be sensitive to the R43Q substitution and positioned on a loop thought to be involved in the channel-pore opening motion²³ (Fig. 1D). Here we show that these interactions are not present in our novel model of the resting state (Fig. 1C) which was based on the structure of a prokaryotic homolog published very recently.²⁴ These new models (i.e., resting and activated states) point out that the $\gamma 2R43$ domain is affected by the motion resulting from $GABA_A$ receptor activation. This observation is consistent with an allosteric effect of the mutation. Moreover, electrophysiological recordings and kinetic analyses have shown that the long-distance effects of $\gamma 2(R43Q)$ substitution extend as far as the transmembrane domains.²⁰ Then, the extracellular domain might have an influence on receptor endocytosis in line with the current views on pentameric ligand-gated ion channels, describing a link between extracellular-, transmembrane-, and intracellular-domains.²⁵⁻²⁷ This also suggests that allosteric drugs in wide clinical use may have an influence on receptor trafficking.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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